

AD-A217 176

REPORT DOCUMENTATION PAGE

1. Unclassified		15. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE		Approved for public release; distribution unlimited.	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
		ARO 26789.1-CH-CF	
6a. NAME OF PERFORMING ORGANIZATION	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION	
American Chemical Society		U. S. Army Research Office	
6c. ADDRESS (City, State, and ZIP Code)		7b. ADDRESS (City, State, and ZIP Code)	
Washington, DC 20036		P. O. Box 12211 Research Triangle Park, NC 27709-2211	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
U. S. Army Research Office		DAAL03-89-G-0043	
8c. ADDRESS (City, State, and ZIP Code)		10. SOURCE OF FUNDING NUMBERS	
P. O. Box 12211 Research Triangle Park, NC 27709-2211		PROGRAM ELEMENT NO.	PROJECT NO.
		TASK NO.	WORK UNIT ACCESSION
11. TITLE (Include Security Classification)			
Symposium on Biosensors			
12. PERSONAL AUTHOR(S)			
William E. Harfield			
13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 6/5/89 TO 6/4/90	November 1989	
16. SUPPLEMENTARY NOTATION			
The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP	
		Biosensors, Symposium, Sensor Technology, Selectivity, Sensors. (SDW)	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)			
The program for the symposium was selected to emphasize the physics, engineering, analytical chemistry, and biochemistry (including immunochemistry) as they exist now, and as they may make contributions to sensors technology in the 1990s. Fundamental is of sensitivity and selectivity were emphasized wherever possible. Keywords:			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT			
<input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			
21. ABSTRACT SECURITY CLASSIFICATION		22a. TELEPHONE (Include Area Code)	
Unclassified		22c. OFFICE SYMBOL	
22b. NAME OF RESPONSIBLE INDIVIDUAL			

DD FORM 1473, 24 MAR

63 APR edition may be used until exhausted.
All other editions are obsolete.SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

90 01 23 026

Final Report
to the
US Army Research Office
on the
Symposium on Biosensors
organized by the
North Carolina Section
American Chemical Society
September 7-9, 1989
Carolina Inn
University of North Carolina
Chapel Hill, North Carolina 27599-3290

Submitted by
William E. Hatfield
Mary Ann Smith Professor of Chemistry
University of North Carolina
Symposium Chairman
North Carolina Section
American Chemical Society

SENSOR ISSUES FOR THE 1990'S

AN INTRODUCTION TO THE NORTH CAROLINA SECTION AMERICAN CHEMICAL SOCIETY SYMPOSIUM BIOSENSORS

WHY SENSORS NOW?


Analytical chemists have done research on, made and used sensors for years. Ion-sensitive and redox-sensitive electrodes have been known and used for 100 years...since the days of Nernst. Membrane-based glass electrodes were invented in 1906; rudimentary ion selective membrane electrodes appeared soon after synthetic, polymer-based ion exchanging materials were invented in 1935. Continuous electrolytic (coulometric) gas analyzers for SO_2 were made by 1949. Practical amperometric sensors have been around at least since the Keidel Cell Moisture Monitor (continuous electrolytic water monitor of the 1950s). The Clark amperometric ambient and dissolved oxygen partial pressure sensor has been known, commercialized and adapted to various biochemical and clinical analyses for many years. High temperature, solid state electrochemical cells have been known from the detailed analyses of Wagner in the 1950s, and applications of defect, ion conducting oxide cells to fuel cells and the sensing of ambient oxygen have been known equally long. Optical chemical sensors (color-forming or bleaching reactions, shifts of absorption maxima by dielectric change or stabilizing reactions with ground states) were made as soon as inexpensive spectrophotometers became available in the late 1930s and especially 1940s. Practical, small physical sensors for temperature, strain, acceleration, velocity and much more, were available during and after World War II.

So...why sensor research now? The answer is compounded from availability of new technology (not specifically chemical) and better communications among engineers, physicists and chemists than ever seemed possible before.

PRINCIPAL ROLE OF NEW TECHNOLOGY

New technology drives interest in, and development of sensors. Only secondarily have specific needs driven development. New technology has allowed many new embodiments of classical ideas for sensors by permitting use of new structures and new input functions to probe the transfer functions of systems (contributions of physicists and electrical engineers), development and use of new materials (contributions by chemists), and development of new formats especially for in vivo applications (contributions by physiologists). Practitioners of the new technologies are pushing sensor research. After 1970 and the discovery of the embryo chemically sensitive semiconductor devices, electrical engineers and solid state physicists took up the chemical sensor field, and continued the physical sensor microminiaturization, especially fabrication of small versions of already practical devices. Applications of the field effect-based sensors per se in microfabricated devices seems very much less important than once thought.

Similarly the contributions of physicists in designing quartz crystal piezoelectric devices have expanded the chemical sensor field greatly, although the concept of mass sensors for ambient adsorb- and absorbable gases using the change in resonant frequency of a bulk quartz crystal was exploited some 20 years ago. The

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forms of devices: thick crystal bulk wave (conventional gas analyzer or film thickness monitor), thick crystal shear wave (quartz microbalance), SAW delay time (Rayleigh wave along surface of a relatively thick crystal exposed to gases or liquids), SAW resonant frequency excited from the under side of the crystal, and very thin quartz films oscillating in complex ways (Lamb waves) are parts of the emerging technology for applications to gas and to liquid samples. Materials with crystals in the 21 space groups without a center of symmetry show changes of dimension with application of an electric field, and conversely. On the same side of irreversible thermodynamic equation is the pyroelectric effect in which a thermal gradient induces a field and a measurable voltage drop. This latter effect is presently used in the dc mode, in contrast with the piezoelectric devices that use ac mode. Many of the same materials show both pyro and piezoelectric effects. However the rules for selecting materials requires knowledge of crystal symmetry. These new devices are exceedingly sensitive to thermal gradients (heat flux) and seem, at this early date, to be a most profitable direction for chemical sensors using thermal effects: adsorption-desorption, solution-dissolution, simple bond formation (of all types)-dissociation enthalpies. This statement does not intend a criticism of temperature sensors such as thermistors whose physics and chemistry remain an interesting field for research on mechanisms of charge conduction and material properties.

The role of optical physics cannot be underestimated for the development of optical absorbance change or luminescence sensors

interrogated by probe beams and/or read by emitted light through optical wave guides: through transparent optical fibers of plastic or glass. Sensors using specially coated optical wave guides, including optical fibers and films that modulate the evanescent wave, depending on chemical reactions with the exterior coating, are another development from advanced technology. The latter technology is particularly difficult for conventionally trained chemists because a new parameter; the refractive index of materials is an important in design as the chemistry of the absorbing or reacting layer. Chemists don't usually know or even think about the optical constants of reactive materials.

Contributions by physiologists and clinical chemists are not so clearly recognized, but very important . . . added in terms of commercialization by large companies and several start-up companies. In vivo optical sensors, the microencapsulated pH sensing dyes called optodes, were conceived and developed at the Max Planck Institute for Systemic Physiology by Lubbers and coworkers. Later a very closely related concept was developed at Livermore Labs by Hirschfeld and coworkers. Conversion and specialization of conventional electrochemical sensors into physiologically useful sensors has been a technological triumph. Long before analytical (and electrochemists) discovered microelectrodes using carbon fibers or microfabricated dots, spots, hemispheres and lines, electrophysiologists had already made 0.1 μ tip capillaries as junction-type reference electrodes (for net membrane potential measurements), and made ion exchanger-filled tips for ion assays within single cells. One must also mention the

leadership role of physiologists and clinical chemists in directing attention of analytical chemists and other sensor designers to the special problems of clinical, intensive care, doctors' offices, and in vivo measurements.

A logical combination of 1) the interfacial potential difference (pd) at an electrolyte/membrane interface (whether generated by faradiac or non-faradiac charge separation processes) and 2) the well irradiation by energetic photons, leads to a new kind of device. The notion is 'light addressable potentiometry'. Whatever the interfacial pd is, it can be perturbed and in some sense measured by generation of charge than partially cancels the local field.

THE PRESENT DIMINISHED ROLE OF CHEMISTRY

For about 20 years many of the current ideas of sensor chemistry have been known and exploited. These include use of chemically modified redox electrode surfaces (the Sharp electrodes); use of enzymes in reactive layers to generate from neutral charge substrate species that can be sensed (potentiometric enzyme electrodes); use of enzymes in amperometric sensors (modifications of Clark oxygen electrodes); use of enzymes and mediators in amperometric sensors (glucose sensors since 1960s); use of mediators in redox reactions, formerly called 'secondary intermediates' in early 1950s ($\text{Br}_2\text{-Br}^-$ for oxidation of electrochemically inert SO_2 , or Mn^{2+} for NO_2); use of selective reagents to generate response specificity (crown ethers and natural and synthetic ionophores in monovalent and divalent ion sensors since 1965); use of selective layers to extract or partition

species into surface layers on sensors (King quartz crystal mass sensors in mid 1960s), are but a few examples.

The new technology, contributed by chemists in recent years, has almost always been a perturbation on existing classical ideas, formats or systems. Electronic-conducting electrodes with surfaces modified with pure ion conductors cannot serve as sensors for non-redox ions since the membrane/electrode interface is blocked (capacitively coupled). However, in the same way that mercury films can accumulate trace metals prior to anodic stripping, ion exchange films can accumulate trace ionic redox species that could be subsequently determined by either anionic or cathodic stripping. Electronic-conducting electrodes, modified with mixed conductors, can be sensors of organic gases that dissolve and increase ion or electron mobilities in a reproducible way. But this is a rather obvious exercise.

More importantly, mixed conductor films may selectively catalyze the surface redox reactions of species that are not themselves electroactive in rapid, reversible ways. Redox enzymes involving coenzymes NAD, FAD, and certain quinones need this catalysis by 'promoter' films. Or, enzyme/coenzymes can be incorporated in mixed conductor films with exciting applications to amperometry of electrochemically inert substrates such as ethanol. At this time, the question of just how effective the enzyme system can be made, remains unanswered. How many turns-over can the enzyme system, mediator, promoter combination sustain? Layered systems of electrode, electron transfer promoter layer (NMPTCNQ-type, ferrocene and other covalently attached layers with

different degrees of flexibility in penetrating to the enzyme active site), enzyme/coenzyme reaction layer, dialysis stabilizing layer, may be less effective than mixed or wired systems. In the latter, covalently attached electron transfer reagents are used to modify the enzyme. The latter is imbedded in a mixed conductor polymer. An unmodified enzyme/coenzyme system can be least partially 3-D wired by mixing it with saturated organic solution of promoters. Extensive literature has been accumulating recently on variations of glucose oxidase chemistry at electrodes especially. But the principles are general and many sensors for neutral species attacked by redox enzymes seem not far away.

Design of open chain and cyclic ionophores for selective extraction of particular ions, has been a major achievement in the synthetic part of chemical sensor research. Not only have new ion-dipole compounds been made (with controlled locations of ether oxygens, carbonyl oxygens and nitrogen centers), but carriers with reversible covalent bond formation have been discovered. The major thrust has been to remove ordinary electrostatic (simple ion exchange) selectivity as the main operating principle that leads to the usual Hofmeister series. Instead selective interactions are emphasized to produce sensors that deviate from Hofmeister sequence to permit analysis of phosphate and bicarbonate, for example, and Li^+ and Mg^{+2} among the cations.

Design of optical sensors has again lagged behind trial and error development. It is clear now that color forming reactions and related classical technology can be adapted to wave guide technology. It is clear that colored hydrophobic complexes and

organic dyes change their absorption coefficients and wavelength of maximum absorption upon changing the composition of the membrane phase through absorption of substances to be detected. However the microscopic design of systems to accomplish a selective color reaction seems to be lacking.

THE CONSUMER ROLE: AN EXAMPLE, BIOTECHNOLOGY, AND MEDICINE

New applications of sensors in biotechnology and in medicine give visibility to the sensor field because the applications may be important, e.g., blood gas analyzers, blood parameters for critical care settings and for doctor's offices (pH, pK^+ , pNa^+ , pCa^{2+} , pCl^- , $pHCO_3^{-}$, BUN, glucose, and hematocrit). But relatively few, if any, new principles for sensors have come from these fields. Instead, the applications to these fields have served to broaden the knowledge of sensor developers, especially in the direction of microsensors, and in vivosensors. Problems such as biocompatibility brings a new dimension to the analytical chemist. Problems such as biocompatibility brings a new dimension to the analytical chemist. Awareness of materials science has a surprising impact on sensor design that has moved the entire field from conventional 'dip' style sensors to much more sophisticated products. The latter allows one to do a better measurement job when 'smaller is better', and when low output impedance is necessarily better than high, for example.

On the other hand, different technologies just because they are new, do not necessarily demand changes in products and processes that are already satisfactory and successful. For example, it is merely interesting to apply 'fast' ion conductors

of Na^+ or reconstituted protein Na^+ -conducting channels in lipid bilayers to construction of sodium ion sensors. The operating principle is simply that a material is selected that has rapid, reversible interfacial ion exchange and ideal permeability (transference number of unity). Glass polymeric silicate-aluminate membranes and passive plasticized membranes containing synthetic neutral carriers or ion exchangers are already satisfactory, stable, rugged, and generally useful.

THE EDUCATION - COMMUNICATION ROLES: Chemists and Other Disciplines

Discovery of new principles seem to have nothing to do with the present widespread interest in sensors. Virtually all of the principles of new sensors have been known from classical notions to electrostatics, thermodynamics and transport (in the case of electrochemical sensors), physical optics, energy schemes of optical transitions for absorption and emission, effects of local environment and reactions on transition energies (in the case of optical sensors), and coupled mechanical-electrical-thermal effects (in the case of mass and thermal flux and other physical sensors). Analytical chemists, especially, have realized the need for sensors and detectors. It has been a subsection of analytical chemical science for many years.

The problem has been, and still is, one of organizing the technologies and corresponding specialists. Developing sensors is largely a communication problem. Communication of technology is one part, but communication of basic chemical materials science has been another. The difficulty has been communication of chemical, electrochemical, and analytical spectroscopic ideas to engineers

and some physicists. However, the barriers are largely down through the aid of workshops, symposia, personal communications and collaborations. The next barrier is the biochemical and immunoscience interface with analytical chemistry. Progress has been made almost exclusively by bioanalytical chemists who are already conversant with theory and practice of these important fields.

In devising the program for this Symposium, tutorials on fundamentals of electrochemical, microelectronic, optical and piezoelectric devices was presented for the edification of all. These included:

Richard P. Buck Electrochemical Sensors and Biosensors; Some
Connections with Optical Chemical Sensors

Jay N. Zemel Microfabricated Chemical Sensors

Raymond E. Dessy Swords into Plowshares - the Way Waveguide,
SAW, and Piezoelectric/Pyroelectric Sensors
Work

CONTROL OF THE CHEMICAL SENSOR FIELD

Chemical sensors make up the majority of new sensors. Chemical, including environmental, biochemical, biotechnological, clinical, and medical applications dominate the field. Yet, microfabrication, microstructure, microelectronic technology is, at least partially, driving research at the present. It should be an important aim of chemists to get control of this field since it

is the chemistry that provides the recognition processes, while the size, shape, and structure of the device are merely parts of the transduction and/or amplification. Otherwise chemical sensors will be relegated to the eighth volume of a series on solid state sensors!

PROPOSED DEFINITIONS: PHYSICAL, CHEMICAL AND MIXED TRANSDUCER DEVICES

An arrangement that passively generates a measureable material property is a chemical transducer device. It incorporates a recognition process that is characteristic of the material at the molecular, chemical level, and it incorporates a transduction into a useful signal. A pure physical transducer device generates and transduces a parameter that does not depend on the chemistry per se, but is a result of the system responding as aggregates of point masses or charges. There must also be some gray areas that defy these categories. The transduction into 'a useful signal' requires external measurement, and may require a perturbation, i.e., an optical absorption change requires a probing light beam. A resistance change requires an applied ac voltage, etc. A voltage generated by a chemical system: a membrane cell or a battery is clearly a chemical sensing device. A thermistor temperature measurements of an arbitrary phase does not reflect the chemistry and so is physical. A thermocouple voltage measurement could conceivably be either chemical or physical: to determine an alloy composition at constant temperature, or to measure temperature with known or calibrated alloy. Certain measurements: vibration, force, displacement, velocity, acceleration, flow rate, volume, time,

3-D structure are most often physical. However chemical effects on displacement or vibration are the basis of piezoelectric devices. Temperature changes from background, optical transmission, absorption, refraction, emission, and scattering changes, magnetic permeability changes, voltage, current, resistance, and capacitance changes are most often the transduction part of chemical sensor devices.

PROPOSED DEFINITIONS: CHEMICAL SENSORS AND BIOSENSORS

Chemical sensors are those that use chemical processes in the recognition and transduction steps. Biosensors are a subsection of chemical sensors that use biological recognition processes. Both are intrinsically 'chemical'.

SENSORS VS SYSTEMS: SELECTIVITY, SENSITIVITY, AMPLIFICATION

Selectivity is a measure of a sensor's ability to discriminate among species of similar chemical character. It is connected with the sensors primary 'recognition' function. On the other hand, the inherent transduction possesses some amplification which is further enhanced by the measuring circuit. Thus, sensitivity which is the measure of low level detectability is connected with amplification. Smart sensors have the amplification 'on board'. But as soon as one attempts to improve selectivity or sensitivity, by additional separation steps or addition hardware, the sensor becomes a system. Once again there can be gray areas when distinguishing sensors from sensor systems. Often it is perfectly clear, e.g., when a whole chromatographic system contains a sensor at a distinguishable point.

SENSORS, DETECTORS, ACTUATORS AND DOSIMETERS

Sensors are chemically reversible devices. When an ambient is presented to the sensor, the interfacial processes are rapid and reversible. Ideally, a response to a step up in measured quantity reaches a steady state value as soon as the external surface concentration reaches the bulk value by usual transport processes. A step down returns the response to a lesser value or to baseline. Continuous cycling of steps up and steps down, of various magnitudes, should develop a steady state calibration response. Some level of hysteresis must be expected and a slow move of base line response may be tolerated and corrected by sequential standard samples. Some sensors are not reversible and, in the worst case, are one-shot detectors that self destruct or become insensitive after a single measurement. Actuators are sensors or detectors combined with an active output. Dosimeters are virtually unsaturable irreversible detectors that continue to accumulate a one directional input signal.

DEVELOPING THE PROGRAM FOR THE SYMPOSIUM ON BIOSENSORS

In view of the concepts and definitions enunciated above, the program was selected to emphasize the physics, engineering, analytical chemistry, and biochemistry (including immunochemistry) as they exist now, and as they may make contributions to sensors technology in the 1990s. Fundamental issues of sensitivity and selectivity were emphasized wherever possible. In addition to the Tutorial Lectures (above), the program included:

MICROELECTRODES AND MICROELECTRONIC DEVICES

Jiri Janata:	Chemically Sensitive Field Effect Transistors
R. Mark Wightman:	Voltammetric Sensors for Neurochemistry

MODIFIED ELECTRODES, AMPEROMETRIC, AND POTENTIOMETRIC SENSORS

- Yoshio Umezawa:** Novel Sensing Membranes for Organic Guest,
Based on the Host Functionalities for Macro-
cyclic Polyamines and Related Compounds
- Philip N. Bartlett:** Applications of Enzymes in Amperometric
Sensors - Problems and Possibilities
- Royce W. Murray:** Electrochemical Reactions, Enzyme Electro-
catalysis, and Immunoassay Reactions to
Hydrogels
- Lemuel B. Wingard:** Amplification Possibilities with Neurorecep-
tor-Based Biosensors

OPTICAL AND ACOUSTIC WAVE-BASED SENSORS

- Masuo Aizawa:** Optical Fiber Electrodes for Electrochemical
Luminescence-based Homogeneous Immunoassay
- Joseph D. Andrade:** Remaining Problems in the Development of
Remote, Continuous, Multi-channel Devices
- Raymond E. Dessy:** A Microelectronic Analytical Toolbox
- George G. Guilbault:** Immobilized Enzyme-based Fiber Optrodes
- Kisholoy Goswami:** Fiber Optic Chemical Sensors (FOCS) - An
Answer to the Need for Small, Specific
Monitors
- W. Rudolf Seitz:** Indicator Substrates for Fiber Optic Chemi-
cal Sensors

The complete program is included in the Abstract Booklet which is attached as Appendix I and the list of participants is attached as Appendix II.

SYMPOSIUM ON BIOSENSORS - September 7-9, 1989
GRANT NO.: DAAL03-89-G-0043
FINANCIAL REPORT

Received from US Army Research Office (7/13/89)		\$1,800.00
To be sent after final reports are filed		<u>200.00</u>
Total		\$2,000.00
Expenditures:		
Printing	\$1,551.98	
Mailing	63.02*	
Advertising	<u>385.00</u>	
Total	\$2,000.00	\$2,000.00

*Remainder of these charges were paid from another source.

Appendix I

SYMPOSIUM ON BIOSENSORS

SEPTEMBER 7-9, 1989

AT THE
UNIVERSITY OF NORTH CAROLINA
AND THE CAROLINA INN

PRESENTED BY THE
NORTH CAROLINA SECTION OF THE
AMERICAN CHEMICAL SOCIETY

PROGRAM FOR SYMPOSIUM ON BIOSENSORS

Thursday, September 7, 1989

Session Chairman: Alvin L. Crumbliss

Venable 207 - UNC Campus

**Tutorials on Fundamentals of Electrochemical, Microelectronic,
Optical and Acoustic Wave - Piezo Electric Sensors**

- 7:15 p.m. Richard P. Buck
Electrochemical Sensors and Biosensors; Some
Connections with Optical Chemical Sensors
- 8:15 p.m. Jay N. Zemel
Microfabricated Chemical Sensors
- 9:15 p.m. Raymond E. Dessy
Swords into Plowshares - the Way Waveguide, SAW
and Piezoelectric/Pyroelectric Sensors Work

Friday, September 8, 1989
Carolina Inn - Old Well Room

Session Chairman: C. Edgar Cook

Microelectrodes and Microelectronic Devices

- 9:00 a.m. Jiri Janata
Chemically Sensitive Field Effect Transistors
- 9:45 a.m. R. Mark Wightman
Voltammetric Sensors for Neurochemistry
- 10:30 a.m. **BREAK - South Parlor**
- Modified Electrodes, Amperometric, and Potentiometric Sensors**
- 10:45 a.m. Yoshio Umezawa
Novel Sensing Membranes for Organic Guest, Based
on the Host Functionalities of Macrocyclic
Polyamines and Related Compounds
- 11:30 a.m. Phillip N. Bartlett
Applications of Enzymes in Amperometric Sensors -
Problems and Possibilities
- 12:15 p.m. **LUNCH BREAK**
- Session Chairman: Brian L. Shigekawa**
- 1:15 p.m. Royce W. Murray
Electrochemical Reactions, Enzyme Electrocata-
lysis, and Immunoassay Reactions on Hydrogels

- 2:00 p.m. Lemuel B. Wingard
Amplification Possibilities with Neuroreceptor-based Biosensors
- 2:45 p.m. Willfred Schramm
Analyte-enzyme Conjugates as Signal Generators in Immunosensors
- 3:30 p.m. **BREAK - South Parlor**
- Optical and Acoustic Wave-based Sensors**
- 3:45 p.m. Masuo Aizawa
Optical Fiber Electrodes for Electrochemical Luminescence-based Homogeneous Immunoassay
- 4:30 p.m. Joseph D. Andrade
Remaining Problems in the Development of Remote, Continuous, Multi-channel Devices
- 5:15 p.m. Adjourn for Poster Session and Social Hour
- 5:30 p.m. Poster Session and Social Hour
Carolina Room, North Parlor and South Parlor
-

Saturday, September 9, 1989
Carolina Inn - Old Well Room
Session Chairman: David Lewis

- 8:45 a.m. Raymond E. Dessy
A Microelectronic Analytical Toolbox
- 9:30 a.m. George G. Gilbault
Immobilized Enzyme-based Fiber Optrodes
- 10:15 a.m. **BREAK - South Parlor**
- 10:30 a.m. Stanley M. Klainer
Fiber Optic Chemical Sensors (FOCS) - An Answer to the Need for Small, Specific Monitors
- 11:15 p.m. W. Rudolf Seitz
Indicator Substrates for Fiber Optic Chemical Sensors
- 12:00 p.m. Adjourn Symposium

SYMPOSIUM ON BIOSENSORS

ABSTRACTS FOR SPEAKER PRESENTATIONS

MICROELECTRODES AND MICROELECTRONIC DEVICES

SOLID STATE POTENTIOMETRIC SENSORS. Jiri Janata, Center for Sensor Technology, University of Utah, Salt Lake City, Utah 84112.

Investigation of solid state devices as chemical sensors over the last fifteen years has led to development of a new type of electroanalytical instrumentation in which the selectivity and amplification are combined and miniaturized. New electrodes for both ionic and electrically neutral species have been developed, the theory of their operation has been formulated and most practical problems related to their preparation have been solved. These sensors include ion sensitive field-effect transistors, enzymatic transistors and metal-insulator-semiconductor structures for measurement of chemical modulation of electron work function. In this paper the status of this research as well as future directions are discussed.

VOLTAMMETRIC SENSORS FOR NEUROCHEMICALS. Jayne Brown, Dorale Wiedemann, David Leszczyszyn, Jeffrey Jankowski and R. Mark Wightman, Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599-3290

Many different types of voltammetrically active chemicals are found in the mammalian brain. One class, neurotransmitters, are small molecules which are secreted by neurons to provide information to adjacent neurons. They provide the major route of communication between neurons in the brain. Those that are easily oxidized are dopamine, serotonin and norepinephrine. Molecular oxygen, which is found in a variety of tissues, but is vital to brain function, is easily reduced.

We have developed carbon-fiber electrodes which have a tip radius of $\sim 10 \mu\text{m}$ which can be inserted into the brain for such measurements. The electrodes are coated with a thin polymer film which can reject electroactive species which may be interferences. In the brain of anesthetized rats dopamine has been detected at submicromolar levels and with subsecond time resolution. The measurements are sufficiently fast that the kinetics and mechanism of the factors which regulate dopamine concentration in the spaces between neurons can be determined. Simultaneously, fluctuations in O_2 concentration have been detected. This technique can be used to learn more about the combined effects of neurotransmission and energy use in the brain. For example, the actions of various drugs on dopamine neurotransmission has been examined.

MODIFIED ELECTRODES, AMPEROMETRIC, AND POTENTIOMETRIC SENSORS

NOVEL SENSING MEMBRANES FOR ORGANIC GUESTS BASED ON THE HOST FUNCTIONALITIES OF MACROCYCLIC POLYAMINES AND RELATED COMPOUNDS. Yoshio Umezawa, Department of Chemistry, Faculty of Science, Hokkaido University, Sapporo 060, JAPAN

Synthetic host compounds constitute a promising class of sensory elements for chemical sensors because of their selective receptor function as well as high chemical stability and synthetic versatility. Although many successful applications of acyclic ligands and crown ether derivatives for cation sensors have been reported, there are still limited examples of relevant sensors for anionic guests and almost no example of those for neutral organic molecules.

Macrocyclic polyamines function as synthetic receptors for organic anions and catecholic compounds in neutral water. Based on these findings, a novel potentiometric sensor containing a lipophilic macrocyclic polyamine $C_{16}H_{33}-[16]aneN_5$ as a sensory element was developed. This sensor was proved to show selective potentiometric response to organic anions such as nucleotides and dicarboxylates. Furthermore, potentiometric response to catechol and related compounds was observed under the pH conditions in which the guests almost exclusively exist in the uncharged forms.

Also, biomimetic "ion-channel sensors" based on host-guest molecular recognition were constructed by incorporating the same kinds of receptor molecules in LB molecular assemblies, deposited directly on solid electrodes. The binding of organic stimulants to these receptors induced marked increase (or decrease) of the ion permeability of the membranes, as detected by cyclic voltammetry using $[Fe(CN)_6]^{4-}$ or $[Ru(bpy)_3]^{2+}$ as a marker ion. Such a mode of response, a model "channel opening (or closing)", can be used as signal transduction/amplification for the sensing of even electro-inactive analytes.

APPLICATIONS OF ENZYMES IN AMPEROMETRIC SENSORS - PROBLEMS AND POSSIBILITIES. Philip N. Bartlett, Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK.

The combination of redox enzyme catalysts and amperometric electrochemistry is an attractive approach to chemical sensing. The successful development of such devices poses a number of problems including those of the integration of the electrode and the biological components, stability, reproducibility and interference. In order to overcome these problems it is necessary to understand the interplay of mass transport and kinetics in amperometric enzyme electrodes and to develop strategies for the development of such electrodes.

A number of different approaches now exist to link the flow of electrons in biological electron transfer reactions to electrodes. These include the use of homogeneous mediators, modified electrodes, organic conducting salt electrodes, and covalent modification of the enzyme itself. In addition electrochemical polymerization can be used to immobilize redox enzymes at electrode surfaces. These topics will be discussed and illustrated by examples from recent work.

ELECTROCHEMICAL REACTIONS, ENZYME ELECTROCATALYSIS, AND IMMUNOASSAY REACTIONS IN HYDROGELS. B. N. Oliver, Louis Coury, J. O. Ekegeze, C. A. Sosnoff, and Royce W. Murray, Kenan Laboratories of Chemistry, University of North Carolina, Chapel Hill, NC 27599-3290 C. Kellar and Mirtha X. Umana, Research Triangle Institute, RTP, NC 27709.

Hydrogels of poly(acrylamide) and poly(ethylene oxide) are convenient and biocompatible media for use as physically rigid solid state voltammetry solvents. Thin gel slices containing supporting electrolyte, buffer components, and other reagents, can be laid atop in-plane patterns of working, reference, and auxiliary electrodes, dried in place and then reswelled with microdroplets containing electroactive species, enzyme substrates, or haptens to observe, respectively, conventional electrochemical voltammetry of the electroactive probe, electrocatalytic currents resulting from electron transfer mediated turnover of an enzyme in the presence of the added substrate, or enhanced electrocatalytic currents resulting from displacement of redox-labeled hapten from an antibody by the added hapten. Examples of such reactions in hydrogels will be given including the mediated oxidation of sulfite by sulfite oxidase, of glucose by glucose oxidase, and enzyme-linked immunoassay of phenytoin. The hydrogel films are potentially useful packaging strategies for electrochemical assays employing microlithographically defined, disposable electrochemical microelectrodes.

AMPLIFICATION POSSIBILITIES WITH NEURORECEPTOR-BASED BIOSENSORS. Lemuel B. Wingard Jr., Department of Pharmacology, University of Pittsburgh School of Medicine, 518 Scaife Hall, Pittsburgh, Pennsylvania 15261.

Neuroreceptors are proteins that normally reside in cell membranes and serve to facilitate communications between the outside and inside of the cell. The extracellular side of the protein has one or more regions that selectively bind certain compounds or classes of compounds. Binding leads to opening of a trans-membrane ion channel or modification of the activity of enzymes that reside in the membrane. One molecule binding to an extracellular site results in hundreds or thousands of ions crossing through the channel or in the turnover of similar numbers of enzyme substrate molecules. The change in intracellular ion concentration or enzyme product concentration then go on to regulate intracellular processes.

Efforts to incorporate neuroreceptor proteins in biosensors are under way with acetylcholine, gamma-aminobutyric acid, and other receptor types and with a variety of possible transduction schemes as well as end uses in mind.

ENZYME-ANALYTE CONJUGATES AS SIGNAL GENERATORS FOR AMPEROMETRIC IMMUNOSENSORS. Willfried Schramm and Se-Hwan Paek, University of Michigan, Reproductive Sciences Program and Bioengineering Program.

For the quantitative determination of analytes with immunosensors, enzyme-analyte conjugates are required which generate an electrochemically active species that can be amperometrically detected. Although enzymes as biocatalysts provide a convenient means for signal amplification, the chemical structure of the synthesized enzyme-analyte conjugate may have a profound effect on the analytical performance of an immunosensor. The following aspects will be reviewed: 1. Stereochemically controlled immobilization of antibodies to expose the idiotypic sites to analytes and analyte-enzyme conjugates. 2. Effect of accessibility of analyte ligand in analyte-enzyme conjugates to antibody binding sites. 3. Immunosensor performance with analyte-enzyme conjugates containing one or more analyte ligand per enzyme molecule. 4. Diffusion of electrochemically active species to the electrode surface and diffusion of analyte-enzyme conjugate to the antibody binding site.

The synthesis, separation, and purification of analyte-enzyme conjugates with a predefined ratio of analyte ligands poses a substantial challenge. However, within the limitations of an immunochemical reaction, the performance of sensors can be manipulated by a judicious choice of appropriate conjugates as signal generators.

OPTICAL AND ACOUSTIC WAVE-BASED SENSORS

OPTICAL FIBER ELECTRODE FOR ELECTROCHEMICAL LUMINESCENCE-BASED HOMOGENEOUS IMMUNOASSAY. Masuo Aizawa, Masaru Tanaka, Yoshihito Ikariyama, and Hiroaki Shinohara, Department of Bioengineering, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152 JAPAN

Homogeneous immunoassay has successfully been established with an optical fiber electrode which is designed for highly sensitive detection of electrochemical luminescence. Antigen(/antibody) may be covalently labeled by such molecules as luminol. Labeled antigen (antibody) is active in electrochemical luminescence with retaining immunochemical activity. Homogeneous immunoassay is thus possible without any separation process by counting photons derived from electrochemical luminescence after immunochemical complexation.

An optical fiber electrode was fabricated by sputtering platinum on fiber-optic (diameter: 2mm, length: 150cm). The platinum layer for the working electrode was connected to lead wire with silver paste, while that for the counter electrode was connected with solder. The contact points were then fixed in place and insulated by sealing with epoxy resin.

A photon counter of Hamamatsu Photomix consisted of a photon counter (Type C767) and a power source (Type 752-01). Electrode potential was controlled with a function generator and potentiostat (Hokuto Denko Co.) against a Ag/AgCl reference electrode. The counter output and the current output from the fiber-optic electrode were digitized (8 bit) and stored on a floppy disk with a NEC microcomputer (Type PC-9801VX2) through a GP-IB interface. The data were processed with a microcomputer to reduce background luminescence.

Electrochemical luminescence (ECL) of luminol was measured with the optical fiber electrode. Cyclic voltammetry of luminol indicated that the cathodic reaction should be followed by the anodic reaction to generate ECL. The optimum potentials for these reactions were determined to be -1.5V for the cathodic reaction and +0.65V vs. Ag/AgCl for the anodic reaction. Furthermore, ECL of luminol in neutral pH was maximum when the potential was controlled at -1.5V for 5 s and stepped to +0.65V for 15 s.

Under the optimum conditions, ECL of luminol was detected with the optical fiber electrode in the concentration range from 10^{-6} to 10^{-4} mol dm⁻³.

We have also studied the role of dissolved oxygen, because the dissolved oxygen may easily be reduced to hydrogen peroxide at a potential of -1.5V. Monitoring of the dissolved oxygen was carried out with a Clark type oxygen electrode. After the removal of dissolved oxygen by bubbling nitrogen gas, little luminescence was observed. This finding led us to perform another potential step excitation from 0V to +0.65V in the presence of hydrogen peroxide. The electrochemical luminescence generated by the application of positive potential of +0.65V was proportional to the concentration of luminol in the concentration range from 10^{-6} to 10^{-4} M in the presence of 2mM hydrogen peroxide.

IMMUNOSENSORS: REMAINING PROBLEMS IN THE DEVELOPMENT OF REMOTE, CONTINUOUS, MULTI-CHANNEL DEVICES. J.D. Andrade, J-M Lin, V. Hlady, J. Herron, D. Christensen, and J. Kopecek, Department of Bioengineering, University of Utah, Salt Lake City, Utah, 84112, (801) 381-4379.

Immunosensors are generally based on the specific high sensitivity recognition and binding of an antigen (Ag) (the analyte of interest) in a complex solution with an immobilized antibody (Ab), usually an IgG or its Fab fragment. The association constant, K_a , of the Ag-Ab binding process generally dictates the sensitivity and dynamic range of the device. The on-rate constant, K_{on} , determines the speed of the assay, while the off-rate constant, K_{off} , controls the dissociation of the complex and thus the response of the device to changes in circulating Ag concentration. $K_a = K_{on} / K_{off}$. The devices work by detecting the amount of Ag bound to the immobilized Ab. Popular detection signals include change in mass, (gravimetry), change in interfacial optical properties (ellipsometry, plasmon resonance, coupling angles for wave guides), fluorescence, and chemiluminescence. In the latter two cases, a label is generally required. Usually a labelled-Ag is delivered or released in the sampling volume and competes with the unlabeled analyte for the available Ab binding sites.

- Problems:**
- 1) Common methods of immobilization of Ab to solid surfaces generally result in a great increase in the dissociation time, making the binding process essentially irreversible, even for low K_a systems;
 - 2) The requirement for a labelled competing Ag complicates the design and development of an immunosensor, because means must be provided for the remote delivery of the competing Ag - this is a particular problem for large protein antigens;
 - 3) The long term stability of immobilized Ab and/or Fab is a potential problem due to slow irreversible denaturation processes, oxidation, and proteolytic processes;
 - 4) Non-specific binding, sample background signals, and compatibility with/inertness to the sampling environment are problems in certain situations.

Solutions: Our approach to each of these problem areas will be described. The talk is basically an update/progress report - see Reference 1-3 for background and earlier work.

References:

1. J.D. Andrade, et al., "On-Line Sensors for Coagulation Proteins," Biomaterials 2 (1988) 76.
2. J.D. Andrade et al., "Fiber Optics Immunodetectors: Sensors of Dosimeters?," SPIE Proc. 712 (1986) 280.
3. J. Ives, et al., "Fiber Optic Fluorescence Immunosensors," Ann. Biotech. Lab., March, 1989.

THE MICROELECTRONIC ANALYTICAL TOOLBOX. Raymond E. Dessy,
Chemistry Department, Virginia Polytechnic Institute and State
University, Blacksburg, VA 24061.

The microelectronic revolution has contributed a number of interesting tools to the armamentarium of the analytical chemist. Many of these weapons are based on serendipitous observations that recognized the deficiencies in physical transducers, noted by physicists or electrical engineers, were starting points for the development of chemical transducers.

SAW devices, designed for electronic filtering, drift as the ambient air conditions change. They make excellent gas sensors.

Optical fibers, used in digital telecommunications, drift as the temperature of the surroundings change. They make excellent biosensors or gas sensors. Simple two-arm and one-arm interferometers provide peroxide/catalase, urea/urease, hydrogen/oxygen, hydrogen/olefin, or methane/oxygen sensor systems.

These same fibers "leak" radiation slightly when used for telecommunication. This leakage can be used to probe the absorbance, fluorescence, and refractive index of the surroundings. The silica surface of the outside of the fiber acts like the inside of a microbore capillary column, and interesting exchange and equilibrium phenomena can also be explored.

Piezoelectric organic films show a pyroelectric effect, making them ideal candidates for inexpensive biosensors, where the enzyme is immobilized on or near the film.

The configuration of these sensors, the necessary support electronics, and typical applications will be presented.

FIBRE OPTIC BASED BIOSENSORS UTILIZING IMMOBILIZED ENZYME SYSTEMS.
Abdel-Latif, M. S. and Guilbault, G. G., University of New Orleans, Department
of Chemistry, New Orleans, Louisiana 70148

Biosensors are attracting the attention of many investigators in the field of biotechnology related research. A biosensor is a device that combines the specificity of a biomaterial (enzymes, antibodies, receptors or even whole cells) with the sensitive and well established bioassays. Biosensors are of potential use in many fields including clinical and biotechnology monitoring. An important class of enzymes results in H_2O_2 production after the reaction with a suitable substrate, and O_2 is consumed. Either H_2O_2 generation or O_2 depletion can be monitored to follow the enzymatic reaction and thus determine the substrate level. Other enzyme systems, like urease and penicillinase, result in a pH change which can be followed using a pH sensitive device. For the many advantages of fiber optic sensing, extensive research efforts have been devoted for the production of reliable, self-contained and sensitive fiber optic biosensors which are capable of in situ monitoring. Results of such an approach for the monitoring of peroxide generating and O_2 consuming biosystems will be presented.

FIBER OPTIC CHEMICAL SENSORS (FOCS) - AN ANSWER TO THE NEED FOR SMALL, SPECIFIC MONITORS - Stanley M. Klainer, Ph.D., Kisholoy Goswami, Ph.D., Dilip K. Dandge, Ph.D. - ST&E, Inc., 1233 Quarry Lane, Suite 145, Pleasanton, CA 94566-8452; Johnny R. Thomas, Ph.D. - FCI, Inc., 3904 Juan Tabo Blvd. N.E., Albuquerque, NM 87111

The need for small, specific, inexpensive sensors has been the driving force behind FOCS development. Finally, after more than a dozen years of research, practical devices are emerging and commercialization is underway.

There are numerous steps that must be considered between starting out with a species or group-specific chemical reaction and ending up with a viable FOCS. These will be discussed as an introduction to the overall ST&E/FCI FOCS development program. FOCS designs and chemistries will be presented based on end uses such as environmental monitoring and medical diagnostics. The types of instrumentation needed to operate the FOCS will be shown. Laboratory calibration and test data will be presented as will field test data for some of the environmental FOCS. Finally, the long-term ST&E/FCI research to commercialization plan will be projected.

INDICATOR SUBSTRATES FOR FIBER OPTIC CHEMICAL SENSORS. W. Rudolf Seitz, Zhang Zhujun, Amy Sommers and Richard Russell, Department of Chemistry, University of New Hampshire, Durham, New Hampshire, 03824

Fiber optic chemical sensors consist of an immobilized indicator phase coupled to a spectrometer through fiber optics. To control the response characteristics of the indicator phase, it is necessary to have a means of controlling both the amount of indicator and the amount of substrate. We are developing systems which allow for independent control of both these parameters. The first step is to chemically couple the indicator of interest to dissolved polymer. The amount of immobilized indicator is then determined by spectrophotometry. If necessary, the immobilization is repeated to increase the amount of indicator. At this point crosslinking reagent is added under conditions such that the rate of crosslinking is slow enough so that it takes several minutes for polymer/indicator/crosslinker to solidify. This allows time to accurately transfer a known volume of substrate to the fiber optic system.

This approach was initially developed using poly(vinyl alcohol) as the polymer, glutaraldehyde as the crosslinking reagent and hydrochloric acid as a catalyst to control the solidification. However, poly(vinyl alcohol) is limited as an indicator substrate because relatively few immobilization chemistries are available for coupling to hydroxyl substrates and because crosslinking with glutaraldehyde requires highly acid conditions which will denature most biomolecules. Therefore, we are developing analogous procedures using poly(ethylenimine) as the polymer and dimethyl adipimidate, a reagent widely used to crosslink proteins. At neutral pHs, dimethyl adipimate crosslinks poly(ethylenimine) forming a clear firm gel.

ABSTRACTS FOR POSTER PRESENTATIONS

A BIOSENSOR BASED ON PYROELECTRIC POLY(VINYLIDENE FLUORIDE) FILMS.

Lawrence M. Arney, Jr., C.N.R.S., 24, avenue du Président Kennedy, 68200 Mulhouse, FRANCE and R. E. Dessy, Virginia Tech Chemistry Department, Blacksburg, Virginia 24061

Thin films of poly(vinylidene fluoride), coated on both surfaces with a thin vapor deposited metal electrode, can be made pyroelectric by suitable stress and poling techniques. Such films make good enthalpic biosensors for use in flow injection analysis systems. First, a bimorph is constructed by placing two sheets of the film face-to-face. This is cut and formed into the side of a flow channel. The common center electrodes are grounded; the outer electrodes provide a sample and reference lead connection. A suitable enzyme is immobilized on the other side of the flowcell. As substrate flows through the cell the heat generated at the biopolymer diffuses to and through the pyroelectric bimorph, giving rise to an electrical signal. The bimorph construction helps reject temperature and pressure effects that are experienced concurrently by the films. The system has been successfully applied to the enzyme catalase measuring concentrations of peroxide.

A THIN-FILM FLEXIBLE MICROELECTRODE ARRAY FOR USE IN ELECTROPHYSIOLOGY RESEARCH

R.B. Asfi¹, S.S. Schiffman², H.T. Nagle¹, J.J. Wortman¹

¹ Department of Electrical and Computer Engineering, North Carolina State University, Raleigh, NC 27695. ² Department of Psychiatry, Duke University Medical Center, Durham, NC 27706.

The design and fabrication of a flexible microelectrode array capable of recording action potentials from single neurons are described. The device consists of three plunge probes with four recording sites on the tip of each probe and is designed to be inserted up to 2.5mm into the brain of a rat with all twelve recording sites active within a nucleus area. The probes are each 0.13mm wide with a center to center spacing of 0.30mm while each of the recording sites are 0.01mm by 0.01mm and arranged on a 0.05mm vertical center to center spacing. The array is fabricated with silver sensing sites on a thin Kapton (polyimide) substrate. Tests on the electrodes are being conducted in electrophysiology experiments at Duke University.

ELECTROCHEMISTRY OF POLYPYRROLE-GLUCOSE OXIDASE ELECTRODE. Daniel Bélanger, Jocelyn Nadreau and Guy Fortier, Département de Chimie, Université du Québec à Montréal, C.P. 8888, succ. A, Montréal, (Québec) Canada H3C 3P8.

We report the electrochemical behavior of a polypyrrole-glucose oxidase, PPy-GOD, electrode. The electrode is prepared by anodic polymerization of pyrrole and GOD with KCl electrolyte on a platinum electrode. The cyclic voltammetry behavior of a PPy-GOD electrode closely resembles that of a PPy-Cl⁻ electrode. With these electrodes the enzyme-catalyzed oxidation of glucose is followed by the electrochemical detection of H₂O₂ at the surface of the underlying platinum electrode. Surprisingly, we found that an amperometric current, corresponding to H₂O₂ oxidation, was observed only after loss of PPy electroactivity. When the first glucose assay was carried out at potential <0.5 V, no amperometric response is detected while for assays at potentials >0.5 V the electrode was generating a response to glucose. Cyclic voltammogram of such electrode recorded immediately after an assay showed PPy electroactivity only for the former. Electroactivity of PPy is lost prior to glucose determination when the PPy-GOD electrode was held at potential >0.5 V for the time necessary to allow the large background current to decrease to a small constant value. On the other hand, for assays at lower potential, the enzymatically generated H₂O₂ is probably oxidizing the PPy layer instead of being oxidized at the underlying Pt electrode. Thus, no amperometric response is detected in this case.

THE POTENTIAL OF FLUORESCENT LIPID MEMBRANES AS TRANSDUCERS IN BIOSENSORS. J. D. Brennan, R. S. Brown, U. J. Krull and C. L. McClintock. Chemical Sensors Group, Department of Chemistry, Brindale Campus, University of Toronto, 3359 Mississauga Rd. W., Mississauga, Ontario, Canada, L5L 1C6

Interactions of selective proteins bound to lipid membranes with chemical stimulants can lead to large alterations in the structure of the supporting membrane. These alterations may be transduced into optical signals via emission from fluorescently labelled lipids in the membrane. The effect on the fluorescence emission from labelled lipid vesicles or monolayers resulting from the interaction of the membrane bound enzyme acetylcholinesterase with the substrate acetylcholine was studied. The hydrolysis of acetylcholine by acetylcholinesterase produced acetic acid which protonated the acidic headgroups in the membrane and resulted in large increases in relative fluorescence intensity. Fluorescence microscopy of monolayer systems provided evidence for a mechanism of fluorescence enhancement which involved changes in the size and distribution of co-existing phases within the monolayer as a result of the pH change. The potential of fluorescent lipid membranes as generic transducers for biosensor development shall be discussed.

STABILIZATION OF FLUORESCENTLY LABELLED MEMBRANE STRUCTURES ONTO OPTICAL FIBERS FOR BIOSENSOR FABRICATION R.S. Brown, J.D. Brennan, S.C. Ferraro and U.J. Krull, Chemical Sensors Group, Erindale College, University of Toronto, 3359 Mississauga Rd., Mississauga, Ontario, CANADA L5L 1C6

An important step in the development of biosensors is the stabilization of a matrix capable of hosting and maintaining the biological activity of selective binding materials such as enzymes, antibodies and molecular receptors. Previous work has described the use of phospholipid vesicles containing fluorescent lipid molecules as generic systems capable of transducing membrane structural alterations. Structural alterations can then be induced by conformational changes associated with molecular receptor/ligand interactions or by pH changes associated with enzyme/substrate reactions. This presentation describes the stabilization of stearic acid monolayer membranes containing selective proteins onto alkylated quartz wafers and optical fibers using Langmuir-Blodgett technology. The stabilized monolayers were shown to maintain fluorescence response to pH changes. Incorporation of acetylcholinesterase resulted in a surface-stabilized system capable of detecting acetylcholine derivatives. The multidimensional nature of the fluorescence signal provided for self calibration and correction for interference by concurrent measurement of intensity, wavelength and lifetime.

AMPEROMETRIC GLUCOSE SENSOR FABRICATED FROM GLUCOSE OXIDASE AND A MEDIATOR CO-IMMOBILIZED ON A COLLOIDAL GOLD HYDROGEL ELECTRODE. A.L. Crumbliss*, R.W. Henkens*, B.S. Kitchell[†], S.C. Perine*, J. Stonehuerner[†], and K.R. Tubergen*, *Department of Chemistry, Duke University, Durham, N.C. 27706 and [†]Enzyme Technology Research Group, 710 W. Main Street, Durham, N.C. 27702

As an approach to the development of a reagentless amperometric enzyme electrode, we have investigated electrodes prepared by co-immobilizing an enzyme and mediator in a hydrogel matrix at the electrode surface. We have developed a prototype glucose-sensing electrode based on glucose oxidase (GOD). Two approaches have been used to fabricate these electrodes: 1) co-immobilization of GOD and the mediator within the hydrogel matrix; and 2) adsorption of GOD on a colloidal gold electrode surface with immobilization of the mediator within the hydrogel matrix coating. Both methods provide satisfactory electrodes. Using a substituted ferrocene mediator an electrocatalytic wave indicative of an EC mechanism is observed by cyclic voltammetric techniques. A glucose sensing electrode with a stable and linear response up to a glucose concentration of 20 mM without interference from oxygen will be described.

THE USE OF METAL ISLAND FILMS TO SUPPORT RADIATIVE SURFACE PLASMONS AS A METHOD OF TRANSDUCING INTERFACIAL EVENTS. Reno F. DeBono, M. Thompson, A.L. Mallon and M.J. Scaini; Chemical Sensors Group, Erindale Campus, University of Toronto, 3359 Mississauga Road North, Mississauga, Ontario, CANADA, L5L 1C6; (416)828-5282

Surface plasmons (SP) are charge density waves which involve the oscillation of surface electron density at a metal interface. The use of SP as the basis for biosensor development has been shown to be feasible with a Kretschman or a grating configuration, but neither of these configurations lend themselves to miniaturization for in vivo sensing. An alternative approach has been employed here based on the observation that the visible absorption spectra of small metallic particles is dominated by the SP resonance of valence electrons. The resonance conditions depend on the size and shape of the metal particle and the local environment in which the particles are embedded. If the local environment is functionalized to provide a degree of chemical selectivity, then subsequent binding of an analyte could result in a change in the optical absorption spectrum. This poster will present the results of our initial experiments which are designed to provide a solid and stable support for the metal particles, optimise deposition conditions and to give an indication of the feasibility of this approach.

A SYSTEM ARCHITECTURE FOR AN INTELLIGENT IMPLANTABLE BIOSENSOR INTERFACE. Kenneth W. Fernald, John J. Paulos, and Tom K. Miller, Department of Electrical and Computer Engineering, North Carolina State University, P.O. Box 7911, Raleigh, North Carolina 27695-7911.

A system architecture for an intelligent implantable biosensor interface and telemetry instrument is presented. The system consists of a modular chip-set interconnected by a synchronous serial bus. A user-programmable microprocessor controls all functions, including sensor maintenance, actuator control and telemetry. Data acquisition is performed by a sensor interface chip which provides programmable gain, bandwidth, and sampling rates for 8 channels of input. Each channel may either be sampled continuously or monitored for a specific event. Additional inputs may be implemented using multiple interface chips. An advanced telemetry unit allows bidirectional communication of both data and commands, while providing an error-detection scheme to ensure integrity. The system presented is currently undergoing hardware implementation at North Carolina State University.

OPTIMIZATION OF A POLYPYRROLE GLUCOSE OXIDASE BIOSENSOR.

Guy Fortier and Daniel Bélanger, Groupe en enzymologie appliquée et fondamentale, Dép. chimie, Université du Québec à Montréal, C.P. 8888, Succ.A, Montréal, QC, Canada, H3C 3P8.

An amperometric glucose biosensor was fabricated by electrochemical polymerization on a platinum surface of pyrrole in the presence of the enzyme glucose oxidase in a KCl solution at a potential of +0.65 V vs SCE. The enzyme was entrapped into polypyrrole film during the electropolymerization process. Glucose responses were measured by potentiostating the enzyme electrode at a potential of 0.7 V vs SCE in order to oxidize the peroxide generated by the oxidation of glucose. Experiments were performed to determine the optimal film thickness and optimal concentrations of pyrrole and glucose oxidase giving the best response to glucose. It was found that concentrations of 0.3 M of pyrrole, 65 U of glucose oxidase and a film thickness of 0.17 μm (75 mC/cm^2) were the optimal parameters for the fabrication of the biosensor. Furthermore, the response of the enzyme electrode to glucose was maximal at pH 6 and at a temperature of 40°C. The activation energy was 41 KJ/mole. The linearity of the enzyme electrode ranged from 1 mM to 7.5 mM of glucose and the kinetic parameters determined were 33.4 mM for the K_m and 7.2 μA for the I_{max} .

APPLICATION OF FIELD EFFECT ELECTRO-OSMOSIS TO SEPARATION BASED SENSORS.

Kuimars Ghowsi and Robert J. Gale, Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803

By coating the outside of a silica capillary by a conductive layer and applying a perpendicular voltage, V_G , across its wall, a novel effect called field effect electro-osmosis can be postulated. It is shown theoretically how this effect can increase the resolution and the selectivity of separation-based sensors. It is also possible to reduce the length of the capillary by several times yet achieve normal resolution. Very fast analyses can be achieved by creating very fast electro-osmosis flow. Because in capillary electrophoresis the optimum resolution for separation is obtained when the electro-osmotic flow is equal and opposite to the electrophoretic migration, an easily automated operation is very likely to produce the best resolution. A new generation of chemical sensors is possible based on metal-insulator electrolyte cv response measurements.

AMPEROMETRIC BIOSENSORS FOR GLUCOSE, LACTATE, AND GLYCOLATE BASED ON REDOX-MODIFIED SILOXANE POLYMERS AND OXIDOREDUCTASES.

P. D. Hale, H. I. Karan, T. Inagaki, H. S. Lee, Y. Okamoto, and T. A. Skotheim, Department of Applied Science, Brookhaven National Laboratory, Upton, New York 11973.

Amperometric biosensors have been developed in which electrical communication between the redox centers of the enzyme and the electrode is achieved via a network of donor-acceptor relays covalently bound to a flexible poly(siloxane) backbone [1]. Polymeric systems containing ferrocene and quinone derivatives have been shown to facilitate electron transfer from several flavin-containing oxidases. Amperometric sensors for glucose, lactate, and glycolate have been studied using cyclic voltammetry and stationary potential measurements, and have been shown to be sensitive to small changes in substrate concentration over a useful range of concentrations. In addition, because of the excellent stability of the siloxane polymers and the fact that the electron-transfer relays are not free to diffuse away from the electrode, these sensors display an excellent response over long periods of time.

[1] P. D. Hale et al, J. Am. Chem. Soc. 111, 3482 (1989).

CHEMICAL SENSORS BASED ON PYROELECTRIC TRANSDUCTION

M. S. Heilich, U. J. Krull, R. S. Brown, R. F. DeBono, Chemical Sensors Group, Dept. of Chemistry, University of Toronto, Erindale College, Mississauga, Ontario, Canada. L5L 1C6 (416)-828-5282

A number of selective chemical interactions at the gas/solid or liquid/solid interface have been studied by examining the characteristic emr absorption spectrum. Upon reaching the excited state these systems may relax via "radiationless de-excitation pathways" resulting in local heating. If the excitation source is pulsed at an appropriate frequency, thermal waves produced by a sub-monolayer coating of chromophoric material can be detected by using a pyroelectric material (PEM). The application of a temperature gradient across a PEM produces an electrical potential which can be monitored if both sides of the PEM are coated with a thin metal film. This forms the basis for a chemical sensor in which one side of the PEM is coated with a chemically selective chromophoric surface. The type of metal coating can be chosen to suit the chemistry being stabilized at the surface. This poster will discuss recent work involving stabilization of sulfur-terminated lipids onto PEM's coated with, and will provide results which demonstrate the potential of this transduction scheme as the basis of a chemical sensor.

Antibody-based Biosensor for Continuous Monitoring

Anne W. Kusterbeck, Gregory A. Wemhoff, and Frances S. Ligler

Bio/Molecular Engineering Branch, Code 6190
Naval Research Laboratory, Washington, DC 20375-5000

We have designed and tested an antibody-based biosensor which operates in continuous flow. Immobilized antibodies are saturated with a fluorescently-labelled antigen and placed in a column with a 200 ul bed volume. Samples containing the chemical or biological molecule to be detected (the antigen) are introduced into the aqueous buffer flow and passed over the column. The sample molecule displaces a proportional amount of the fluorescently-labelled antigen, and a signal is generated downstream. The reaction takes place at a flow rate of 0.5 ml/min and has shown a preliminary level of detection in 200 nM range. This type of sensor has practical applications for the detection of small molecules and monitoring a continuous stream for the presence of the analyte of interest.

RAPID FILTRATION-BASED DETERMINATION PERFORMED WITH A SILICON BIOSENSOR

M. Lucas^a, S. Alter^b, G. Kirk^c, M. Huntington^a, F. Regina^a, M. Ballman^b, J. Bolts^a

We present a method in which nanogram quantities of small or large molecules are detected in 1-2 minutes using enzyme-labeled antibodies (Ab-E). An initial immunochemical reaction is performed which results in the capture of Ab-E on a porous, affinity-labeled membrane. A silicon-based Light Addressable Potentiometric Sensor (LAPS) is then used to detect changes in pH caused by the activity of the enzymes on the membrane surface. Two assay configurations are demonstrated using low and high molecular weight analytes. T2 mycotoxin (MW 462) is detected using a displacement reaction in which sample T2 displaces affinity-labeled T2 from binding sites on urease-labeled antibodies. A protein (MW 30,000) is detected using a sandwich format in which the protein complexes with affinity-labeled and urease-labeled antibodies in solution. As little as 2 ug of T2 or 1 ng of protein may be detected within 1-2 minutes. Strategies to optimize assay sensitivity and dynamic range are presented. Also discussed is a mechanical design to automate and precisely time all steps of the entire analysis.

(a) Allied-Signal, Inc., Morristown, NJ; (b) Environmental Technologies Group, Inc., Baltimore, MD; (c) Molecular Devices Corp., Menlo Park, CA

Electrical Conductivity Measurements of Lipid Films Exposed to Iodine Vapor. Gregory E. Madison, Gordon Jendrasiak, Ralph Smith, East Carolina University Medical School, and Thomas McIntosh, Duke University Medical School. Lipids in the solid state (dry lipids) deposited between two electrodes are found to have an increase in current by as much as 10 orders of magnitude when exposed to iodine vapor. Using an iodine-Diethylene Glycol solution, the relative iodine vapor pressure (RIVP) to which the lipid is exposed can be controlled, thereby regulating the conductivity through the lipid. Two lipids being investigated are Egg Phosphatidylcholine (EPC) and Dipalmitoyl Phosphatidylcholine (DPPC). These lipids have the same polar head group, with different degrees of hydrocarbon chain saturation. For EPC, the conductivity range is found to be $<10^{-14} \Omega^{-1}\text{cm}^{-1}$ at 0% RIVP to $10^{-3} \Omega^{-1}\text{cm}^{-1}$ at 100% RIVP. For DPPC, the conductivity range is $<10^{-14} \Omega^{-1}\text{cm}^{-1}$ at 0% RIVP to $10^{-6} \Omega^{-1}\text{cm}^{-1}$ at 100% RIVP. Visible-UV Spectrophotometric measurements show a conversion of I_2 to I_3^- as the iodine vapors come into contact with the lipids. A complete conversion of I_2 to I_3^- over a 48-hour period is found for EPC, while DPPC shows much less I_3^- formation over the same time period with a substantial amount of I_2 remaining. EPC is found to contain one I_3^- molecule per lipid molecule upon saturation, whereas DPPC contain one I_3^- molecule per 8 lipid molecules. X-Ray diffraction data reveals the iodine is absorbed between the lipid bilayers, increasing the repeat period by about 0.8nm. EPC repeat periods, however, decrease by about 0.4nm, indicating an interaction with the double bond in the fatty acid chain. DPPC is found to have a lower electrical activation energy than EPC, suggesting that the conduction process across the polar heads is effected by the degree of unsaturation. A model will be suggested to explain the electrical activity of the lipid-iodine system. Increased conductivity shows possible applications as an iodine sensor. This work was sponsored through a grant by the North Carolina Biotechnology Center.

THE SELECTIVE BINDING OF SUBSTITUTED NITROBENZENES TO 3-AMINOPROPYLTRIETHOXYSILANE STUDIED BY PIEZOELECTRIC CRYSTAL SENSORS AND MOLECULAR MODELLING. W.M. Heckl, F.M. Marassi, K.M.R. Kallury, D. Stone, M. Thompson, Department of Chemistry, University of Toronto, 80 St. George St., Toronto, Ontario, M5S 1A1, Canada,

A 3-aminopropyltriethoxysilane (APTES)-coated surface acoustic wave sensor was observed to behave selectively towards substituted nitrobenzene molecules. The relative binding affinities were measured by analysing the frequency shift of the crystal with time, and were found to range from completely reversible to irreversible. A molecular orbital study using the AM1 method was performed in order to understand the nature of the noncovalent binding interaction between APTES and the test molecules. The results of this study indicate that hydrogen-bonding and dipole potentials are the major factors responsible for the observed selectivity.

Design Calculations for a Chemiluminescent Immobilized Enzyme Photoelectrode

David F. Ollis and Robert Fletcher
Chemical Engineering Department
North Carolina State University
Raleigh, NC 27695-7905

An engineering analysis for an immobilized enzyme chemiluminescent photoelectrode is presented. The device considered consists of an immobilized enzyme membrane placed in front of an appropriate photodetector. Phenomena included in the analysis are solution-to-membrane mass transfer, enzyme loading and catalyzed reaction within the porous membrane, light generation and propagation to the photodetector surface. Material properties such as membrane optical density and enzyme stability are considered.

Presentation: NC ACS, Chapel Hill, 9/8-9/89

THE SILICON MICROPHYSIOMETER: DETECTION OF BIOLOGICAL EFFECTS OF CHEMICAL AND BIOCHEMICAL AGENTS BY ALTERATIONS OF CELLULAR METABOLIC RATE.

J.W. Parce, G.B. Sigal, K.M. Kercso, J.C. Owicki, and V.C. Muir,
Molecular Devices Corporation, 4700 Bohannon Drive, Menlo Park, California 94025.

Using a biosensor comprising the light-addressable potentiometric sensor [Hafeman *et al.* (1988) *Science* 240:1182] and cells in a flow chamber, we have determined that cellular metabolic rates can be detectably altered by many agents, including cytotoxic chemicals and ligands for several types of cellular receptors. In this silicon microphysiometer a 100 μm layer of medium flows across a 1 cm^2 monolayer of adherent eukaryotic cells. Alternatively, non-adherent cells are retained in wells that are micro-machined into the silicon sensor. Signals are obtained from 1 mm^2 regions within the flow chamber, corresponding to about 1000 cells in 100 nL. When flow is temporarily interrupted, production of acidic metabolites slightly decreases the pH of the medium. This process is detected by the sensor, which forms one surface of the chamber. The rate of acidification is a measure of overall metabolic activity. Resumption of flow introduces fresh medium and resets the pH for subsequent measurements, which can be made repetitively and non-destructively for days on the same population of cells. Metabolic rates as low as 10^6 - 10^7 H^+ $\text{cell}^{-1}\text{sec}^{-1}$ can be measured reliably. At present, the temporal and spatial resolutions are ~ 30 sec and < 1 mm. The device is automatable and suitable for basic science and technology. Supported in part by the U.S. Army CRDEC and DARPA, ARO contract DAAL03-86-C-0009.

INTERACTION OF IgG WITH Fc RECEPTORS IN SUBSTRATE-SUPPORTED PLANAR MEMBRANES MEASURED BY TOTAL INTERNAL REFLECTION FLUORESCENCE MICROSCOPY Claudia L. Foglitsch and Nancy L. Thompson, Department of Chemistry, University of North Carolina at Chapel Hill, CB #3290, Chapel Hill, NC 27599

A procedure for constructing substrate-supported planar membranes using membrane fragments isolated from the macrophage-related cell line J774A.1 is described. Total internal reflection (TIR) fluorescence microscopy is employed to demonstrate that fluorescently-labelled Fab fragments of a monoclonal antibody (2.4G2) with specificity for a murine macrophage cell-surface receptor for IgG (moFc_{RII}) bind to the planar model membranes. These measurements show that the planar membranes contain moFc_{RII}, yield a value for the association constant of 2.4G2 Fab fragments with moFc_{RII} equal to $(9.6 \pm 0.4) \times 10^8 \text{ M}^{-1}$ and indicate that the surface density of reconstituted moFc_{RII} is $\sim 60 \text{ molecules}/\mu\text{m}^2$. In addition, TIR fluorescence microscopy is used to investigate the Fc-mediated competition of unlabelled, polyclonal murine IgG with labelled 2.4G2 Fab fragments for moFc_{RII} in the planar membranes. These measurements indicate that $\sim 80\%$ of the reconstituted moFc_{RII} recognized by 2.4G2 Fab fragments also retain murine IgG Fc-binding activity and yield a value for the association constant of polyclonal murine IgG with moFc_{RII} equal to $3\text{-}7 \times 10^5 \text{ M}^{-1}$. This work represents one of the first applications of TIR fluorescence microscopy to specific ligand-receptor interactions and demonstrates a relatively simple approach for reconstituting biologically active receptor proteins on to solid supports.

IMPROVEMENTS TO THE STABILITY OF pH MEMBRANE ELECTRODES, R. P. Buck, R. P. Kusy, K. A. Reinbold, Department of Chemistry and Curriculum in Biomedical Engineering and Mathematics, University of North Carolina at Chapel Hill, Chapel Hill, N. C. 27599.

Stability of our pH microelectrodes, which are presently used for in vivo cardiac experiments, is low. The instability of the membrane, which is composed of a plasticizer (DOS) and a polymer (Aldrich HMW poly vinyl chloride) in a 2:1 ratio with the proton carrier (tridodecylamine), manifests itself in short operating and shelf lives. Instability can be caused by: ripping of the membrane upon insertion into cardiac tissue, thus shorting out the electrode; degradation and leaching out of the proton carrier, decreasing the number of sites available for proton transfer; leaching out of the plasticizer, leading to an increase in resistance by impeding ionic diffusion; and attack by tissue antibodies, destroying the membrane or forming a highly resistant surface film. Impedance measurements, mechanical puncture tests, and thermogravimetric analyses were performed. Materials were optimally chosen to give a low resistance, highly durable, and more stable electrode.

A NICOTINIC RECEPTOR OPTICAL BIOSENSOR. K. Rogers, *D. Richman and M. Eldefrawi, University of Maryland School of Medicine, Dept. of Pharmacol. and Exp. Ther., Baltimore, Maryland 21201. *University of Chicago, Dept. of Neurology, Chicago, IL 60637.

Acetylcholine receptor protein noncovalently immobilized on quartz fibers was used to detect fluorescein isothiocyanate (FITC) labelled receptor ligands (e.g. α -bungarotoxin [α -BGT] and antireceptor antibodies). The optical signal was generated by the fluorescent probe which bound to the receptor protein coating the quartz fiber. The density of immobilized receptor sites was dependent on reaction time, pH, and concentration of the receptor protein in the coupling medium. The receptor biosensor could detect pmol levels of FITC- α -neurotoxins. FITC- α -BGT binding was inhibited by nicotinic receptor agonists and antagonists in a manner consistent with their known receptor affinities. This receptor biosensor was also extremely sensitive in detecting antireceptor antibodies (< 40 fmol of monoclonal antibody MAb 132). Preincubation of receptor fibers for 60 min with sera obtained from myasthenia patients, which contain Ab against the receptor, did not block binding of the MAb. Supported in part by DOD Grant no. DDAA15-89-C-0007 to M.E..

THE DEVELOPMENT OF A CHEMICAL SENSOR BASED ON FORSTER ENERGY TRANSFER.

D.A. Russell, R.S. Brown, M.J. Scaini and U.J. Krull. Chemical Sensors Group, Erindale College, University of Toronto, 3359 Mississauga Rd., Mississauga, Ontario, CANADA, L5L 1C6.

In 1948 Forster observed that fluorescence emission from fluorophores in proximity to a metal surface was quenched. The possibility of utilizing this phenomenon for the transduction of interfacial chemical events is of current interest in our laboratories. For this investigation thin films of chromium followed by gold were evaporated onto substrates of silicon wafers and glass slides. A monolayer of the fluorophore, fluorescein isothiocyanate (FITC), was bound to the gold surface via self-assembly of the isothiocyanate group. Our research has shown that no fluorescence is observed from the FITC after excitation. It is thought that the energy from the excited state is coupling into the conduction band electrons of the gold. Therefore the resistance or conductance of the gold film could be affected by this relaxation mechanism. The results from our investigations will be reported in this poster.

Covalent Attachment of Proteins to Quartz and Silicon with Retention of Biological Activity. Elaine T. Vandenberg, U.J. Krull and M. Thompson. Chemical Sensors Group, University of Toronto - Erindale Campus, 3359 Mississauga Rd, Mississauga, Ont, Canada, L5L 1C6.

Enzymes, antibodies and molecular receptors have been used to provide biochemical selectivity in biosensors. Covalent binding of such proteins to the surface of a transducer is desirable for longevity and reproducibility of device function. Quartz and silicon substrates (ie. models of the surfaces of fiber optic and electrochemical sensors) were derivitized with 3-aminopropyltriethoxysilane followed by glutaraldehyde or tresyl chloride and then protein. The density of protein on silicon surfaces was less than on quartz and was not sensitive to the activation method. The activity of bound antibody was 10 - 25 %. Immobilized antibody-antigen complex and enzymes were characterized by contact angle measurements, ellipsometry, radioactivity, ESCA and fluorescence wavelength, intensity and lifetime.

DEVELOPMENT OF NEURON-BASED BIOSENSORS. R. S. Skeen, W. S. Kisaalita, B. J. Van Wie, S. J. Fung*, C. D. Barnes*, Chemical Engineering Department, Washington State University, Pullman, WA 99164-2710, *Department of Veterinary and Comparative Anatomy, Pharmacology & Physiology, Washington State University, Pullman, WA 99164-6520.

Results in the development of a chemical sensor for the neurotransmitter serotonin which use living neurons are reported. The exposure of identified neurons from the visceral ganglia of the pond snail *Limnaea stagnalis* to serotonin has revealed a concentration dependent increase in the rate of spontaneous action potential generation, over a concentration range of four orders of magnitude. The serotonin induced change is both reversible and repeatable. Data are also presented which indicate that after extended exposure to serotonin the neuron undergoes an adaptive process and its sensitivity diminishes. These findings are discussed in relation to the development of a neuron based chemical sensor.

ELECTROCHEMICAL BIOSENSORS

H. V. Venkatesetty
 HONEYWELL INC.
 Sensor and Systems Development Center
 10701 Lyndale Avenue South
 Bloomington, MN 55420

There is growing interest in developing low-cost and reliable sensors for rapid determination of several chemical species present in blood. Some of the more frequently required determinations in clinical analyses are the partial pressure of carbon dioxide ($p\text{CO}_2$), oxygen ($p\text{O}_2$), hydrogen ion concentration (pH), potassium ion (K^+) and glucose. The three critical parameters to be monitored during heart-lung bypass surgery and for the critical emergency care environment are the $p\text{CO}_2$, $p\text{O}_2$ and pH in the blood. In this paper novel amperometric electrochemical sensors based on nonaqueous electrolyte solutions developed recently at Honeywell for monitoring partial pressures of carbon dioxide and oxygen in blood will be described. The experimental data obtained with these cells using saline solutions saturated with carbon dioxide and carbon dioxide standard solution titrated with carbon dioxide buffer solution will be presented.

THE APPLICABILITY OF METHYL 2-OXOCYCLOALKANOATES AS SURFACE BINDING AGENTS.

K. R. Kallury, U. J. Krull, M. Thompson, S. J. Vigmond, Dept. of Chemistry, University of Toronto, 80 St. George St., Toronto, Ontario, Canada M5S 1A1.

In an effort to construct a surface that can display a degree of molecular recognition through non-covalent interactions, a series of methyl 2-oxocycloalkanoates are being investigated. Results from studies using FTIR, ^1H - and ^{13}C -NMR and MS indicate a preference for the keto form amongst the odd-membered and the enol form amongst even-membered carbocycles of the title series in solution. The potential of these bifunctional carbocycles to act as selective hosts are examined with respect to their keto-enol equilibria, dynamics and structural characteristics determined from AM1 optimizations. Preliminary work reporting on the immobilization of these compounds to a polymeric substrate will also be presented.

BIOSENSORS AND THE EXCITON MECHANISM OF SUPERCONDUCTIVITY AT 310 K (HUMAN BODY TEMPERATURE). James O. Williams and John F. Welsh, Jr., Department of Physical and Earth Sciences, Fayetteville State University, Fayetteville, North Carolina, 28301.

The issues which are raised in nonequilibrium superconductivity as this relates to the exciton mechanism of superconductivity or the role of excitons in materials technology of such magnetic materials as metalloproteins are very important for the understanding of superconducting devices. Quasiparticle injection by tunneling is central to the operation of both the superconducting transistor, the quiteron and three-terminal edge junctions. These are three-terminal superconducting devices of a type which may be very useful in the practical application of superconducting digital electronics, i.e., for the superconducting computer. Since we have accumulated data indicating that metalloproteins are semiconductors, it is now possible to synthesize nanocomputers from proteins and organic molecules or miniaturized electronics which will produce computers, whose elements are molecules that may even reproduce themselves; i.e., replace the transistors and other devices of solid state electronics with molecular functional groups, either organic or inorganic, that are constructed to exhibit the appropriate electrical behavior when hooked together in networks. These tiny computers using the Mott excitonic mechanism of semiconductivity can be made to superconduct at human body temperature (310 K).

TRANSITION METAL ENCAPSULATION BY "METALLOCROWN ETHERS". M. S. Lah¹, M. L. Kirk²
W. E. Hatfield¹ and V. L. Pecoraro¹, Departments of Chemistry, University of
Michigan, Ann Arbor, MI¹ and University of North Carolina, Chapel Hill, NC².

In this contribution we present a new class of metal sequestering agent called metallocrown ethers. Unlike previously described crown sequestering agents (e.g., those with sulfur or nitrogen substitution for the ethereal oxygens to confer greater selectivity or affinity for transition metal ions), the metallocrowns are formed by substituting a trivalent transition metal and nitrogen into the analogous carbon positions of 9-crown-3 or 12-crown-4 using the ligand salicylhydroxamic acid as a template. One then uses ethereal like oxygens to bind divalent or trivalent transition metal ions. Thus, one obtains two levels of specificity for transition metal ions based on selectivity of the cluster forming the ring and then the metal being encapsulated by the metallocrown core. We describe the properties of the V(V) complex, $[V(V)O(Salicylhydroximato)(CH_3OH)]_3$, which forms the 9-crown-3 structure, the Fe^{3+} (9-crown-3), $Fe(III)[Fe(III)(Salicylhydroximato)(CH_3OH)(Acetate)]_3 \cdot CH_3OH$, and two M^{n+} (12-crown-4) complexes, $Fe(H_2O)_6[Fe(III)[Fe(III)(Salicylhydroximato)(sulfato)_{0.5}(CH_3OH)_{1.5}]_4$ and $Mn(II)[Mn(III)-(Salicylhydroximato)(Acetato)_{0.5}(DMF)_{1.5}]_4$.

THIN-LAYER CHAMBER-TYPE BIOSENSORS. Bernard H. Schneider and Otto J. Prohaska,
Department of Biomedical Engineering, Case Western Reserve University, Cleveland,
Ohio 44106.

Amperometric biosensors for glucose and lactate were realized by the immobilization of the enzymes glucose oxidase and lactate oxidase respectively inside the thin-layer volume of chamber-type oxygen sensors. Voltammetric measurements with these miniaturized sensors were not affected by stirring of the external bulk solution, which has important implications for *in vivo* medical applications. Chamber sensors incorporating glucose oxidase entrapped by an agarose matrix demonstrated the same rapid response time as the base sensor of about 10 seconds. The response of these glucose sensors was controlled by oxygen diffusion within the thin-layer chamber volume. By coating chamber sensors, which contained agarose-entrapped lactate oxidase with an additional layer of cellulose acetate, lactate sensors which responded up to 10 mM lactate were achieved. These early results demonstrate the suitability of chamber-type sensors for the development of biosensors on the sub-millimeter scale.

ORGANIZING COMMITTEE AND SPONSORS

SYMPOSIUM ON BIOSENSORS - ORGANIZING COMMITTEE AND STAFF

Dr. William E. Hatfield, Symposium Committee Chairman
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Dr. Richard P. Buck
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Dr. Edmond F. Bowden
Department of Chemistry - Box 8204
North Carolina State University
Raleigh, NC 27695

Dr. Mirtha Umana
Consultant
6823 Falconbridge Road
Chapel Hill, NC 27514

Mrs. Linda Caulder
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Mrs. Evelyn Kidd
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

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P. O. Box 13547
Research Triangle Park, North Carolina 27709

North Carolina Section of the American Chemical Society

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P. O. Box 12211
Research Triangle Park, North Carolina 27709

We express our thanks to these sponsors whose generous support has made the Symposium on Biosensors possible.

Appendix II

BIOSENSOR SYMPOSIUM ATTENDEES
September 7-9, 1989

Akouete Aghodjan
Department of Chemistry
East Carolina University
Greenville, NC 27834

Professor Masuo Aizawa*
Department of Bioengineering
Faculty of Engineering
Tokyo Institute of Technology
Ookayama, Tokyo 152
JAPAN

Stephen C. Alter
Environmental Technologies Group
1400 Taylor Avenue - P. O. Box 9840
Baltimore, MD 21224-9840

S. Michael Angel
Lawrence Livermore National Laboratory
P. O. Box 5507, L-524
Livermore, CA 94550

Professor Joseph Andrade*
Department of Bioengineering
M.E.B. 2480
University of Utah
Salt Lake City, UT 84112

Bruce Ash
Department of Electrical and Computer Engineering
232 Daniels Hall - Box 7911
North Carolina State University
Raleigh, NC 27695-7911

Carleton J. Barbour
Department of Chemistry - CB 3290
University of North Carolina-Chapel Hill
Chapel Hill, NC 27599-3290

Joan E. Bartelt
Department of Chemistry - CB 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Professor Philip N. Bartlett*
Department of Chemistry
University of Warwick
Coventry CV4 7AL
ENGLAND

Charles I. Beck
5215 Mountain View Road
Winston-Salem, NC 27104

Daniel Belanger
Departement de Chimie
Universite du Quebec a Montreal
C.P. 8888, succ. A.
Montreal, Quebec, CANADA H3C 3P8

Robert Bereman
Department of Chemistry - Box 8201
North Carolina State University
Raleigh, NC 27695-8201

C. Randolph Bock
Becton Dickinson Research Center
P. O. Box 12016
Research Triangle Park, NC 27709

Edmond F. Bowden
Department of Chemistry - Box 8204
North Carolina State University
Raleigh, NC 27695

John Brennan
Chemical Sensors Group - Department of Chemistry
University of Toronto - Erindale Campus
3359 Mississauga Road
Mississauga, Ontario, CANADA L5L 1C6

R. Stephen Brown
Chemical Sensors Group - Department of Chemistry
University of Toronto - Erindale Campus
3359 Mississauga Road
Mississauga, Ontario, CANADA L5L 1C6

Professor Richard P. Buck*
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Brent R. Busey
U. S. Army Chemical Research Development
and Engineering Center
ATTN: SMCCR-DDT
Aberdeen Proving Ground, MD 21010-5423

Robert Casper
Glaxo, Inc.
5 Moore Drive - P. O. Box 13398
Research Triangle Park, NC 27709

Lowry Caudill
Glaxo, Inc.
1035 Swabia Court, CNII Room 11
Morrisville, NC 27560

Sachiko Sakura Chapman
Department of Chemistry - CB 3290
University of North Carolina
Chapel Hill, NC 27599-3290

C. Ed Cook**
Research Triangle Institute
P. O. Box 12194
Research Triangle Park, NC 27709

Louis A. Coury, Jr.
Department of Chemistry - CB 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Gloria J. Covington
Environmental Technologies Group
1400 Taylor Avenue - P. O. Box 9840
Baltimore, MD 21284-9840

A. L. Crumbliss
Department of Chemistry
Duke University
Durham, NC 27706

Darla Deanton
Diagnostics - Boehringer Mannheim Corporation
P. O. Box 50100
Indianapolis, IN 46250

Reno F. De Bono
Chemical Sensors Group - Department of Chemistry
University of Toronto - Erindale Campus
3359 Mississauga Road
Mississauga, Ontario, CANADA L5L 1C6

Professor Raymond E. Dessy*
Department of Chemistry
Virginia Polytechnic Institute and
State University
Blacksburg, VA 24061

Angelos Dovletoglou
Department of Chemistry - CB 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Arthur B. Ellis
Department of Chemistry
University of Wisconsin-Madison
Madison, WI 53706

Kerin Faulkner
Department of Chemistry - Gross Chemical Labs
Duke University
Durham, NC 27706

Ken Fernald
Department of Electrical and Computer Engineering
North Carolina State University
P. O. Box 7911
Raleigh, NC 27695-7911

Gary J. Foley
Director, AREAL
U.S. Environmental Protection Agency
MD 75
Research Triangle Park, NC 27711

Dr. Guy Fortier
Département de chimie
Université du Québec à Montréal
C.P. 8888, Succ. A
Montréal, Québec, CANADA H3C 3P8
James E. Francese
Department of Chemical Engineering, BF-10
University of Washington
Seattle, WA 98195

Mike Franklin
Glaxo, Inc.
1035 Swabia Court, CNII Room 11
Morrisville, NC 27560

Kiumars Ghowsi
Chemistry Department
Louisiana State University
Baton Rouge, LA 70803

Margaret Goldberg
Research Triangle Institute
P. O. Box 12194
Research Triangle Park, NC 27709

Dr. Kisholoy Goswami*
ST&E, Inc.
1233 Quarry Lane, Suite 145
Pleasanton, CA 94566-8452

Professor George G. Guilbault*
Department of Chemistry
University of New Orleans
New Orleans, LA 70148

William E. Hatfield
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Alan K. Hauser
Department of Chemical Engineering
University of Texas
Austin, TX 78712-1062

John L. Haynes
Becton Dickinson Research Center
P. O. Box 12016
Research Triangle Park, NC 27709

Michael Heimlich
Chemical Sensors Group - Department of Chemistry
University of Toronto - Erindale Campus
3359 Mississauga Road
Mississauga, Ontario, CANADA L5L 1C6

Robert W. Henkens
Department of Chemistry
Duke University
Durham, NC 27706

Randall A. Hoke
Becton Dickinson Research Center
21 Davis Drive
Research Triangle Park, NC 27709

Brisco Howard
Glaxo, Inc.
1035 Swabia Court, CHII Room 11
Morrisville, NC 27560

Professor Jiri Janata*
Department of Materials Science
University of Utah
Salt Lake City, UT 84112

Dr. Timothy Karpetsky
Environmental Technologies Group, Inc.
1400 Taylor Avenue
Baltimore, MD 2184-9840

Martha Kline
Department of Chemistry - CB 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Anthony Klos
3044 Farrior Road
Raleigh, NC 27607

Anne W. Kusterbeck
64228 Quincy Place
Falls Church, VA 22042

Steve Lackie
ORD, Inc.
P. O. Box 148
North Salem, NH 03073

Robert E. Lee
AREAL, MRDD (MD 78A)
U. S. Environmental Protection Agency
Research Triangle Park, NC 27711

Gabor B. Levy
International Scientific Communications, Inc.
30 Controls Drive - P. O. Box 80
Shelton, CT 06484-0870

David Lewis**
Lord Corporation
Thomas Lord Research Center
P. O. Box 8225
Cary, NC 27512-8225

Chia-yu Li
Department of Chemistry
East Carolina University
Greenville, NC 27858

Mariann E. Lucas
Allied-Signal, Inc. - Biotechnology Department
P. O. Box 1087R
Morristown, NJ 07962

Robert McEnroe
Boehringer Mannheim Corporation - Diagnostics
P. O. Box 50100
Indianapolis, IN 46250

R. A. McGill
Department of Chemistry
University of Alabama
Huntsville, AL 35899

Karen McLachlan
Department of Chemistry
Duke University
Durham, NC 27706

Gregory E. Madison
East Carolina University Medical School
East Carolina University
Greenville, NC 27834

James Mapes
Becton Dickinson Research Center
21 Davis Drive
Research Triangle Park, NC 27709

Francesca M. Marassi
Department of Chemistry
University of Toronto
80 St. George Street
Toronto, Ontario M5S 1A1 CANADA

A. Margaritis
Department of Chemical and Biochemical Engineering
University of Western Ontario
London, Ontario, CANADA N6A 5B9

Larry Margerum
Clorox Technical Center
P. O. Box 493
Pleasanton, CA 94583

Patrick D. Mize
Becton Dickinson Research Center
21 Davis Drive
Research Triangle Park, NC 27711

Eugene Mizusawa
Clorox
P. O. Box 493
Pleasanton, CA 94566

Professor Royce W. Murray*
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Matthew Musho
Miles, Inc.
P. O. Box 70
Elkhart, IN 46515

Acharan S. Narula
Narula Research
1315 Morrene Road, Suite 31-F
Durham, NC 27705

Beverly Nickerson
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

David F. Ollis
Department of Chemical Engineering - Box 7905
North Carolina State University
Raleigh, NC 27695

John C. Owicki
Molecular Devices Corporation
4700 Bohannon Drive
Menlo Park, CA 94025

Alan Parr
Glaxo, Inc.
5 Moore Drive - P. O. Box 13398
Research Triangle Park, NC 27709

Vincent Pecoraro
Department of Chemistry
University of Michigan
Ann Arbor, MI 48109

Sherry C. Perine
Department of Chemistry
Duke University
Durham, NC 27706

Claudia L. Poglitsch
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Kirk A. Reinbold
Curriculum in Biomedical Engineering/Math
Campus Box 7575
University of North Carolina
Chapel Hill, NC 27599-7575

John H. Reynolds
R. J. Reynolds Tobacco Company
Bowman Gray Technical Center
Winston-Salem, NC 27102

David A. Russell
Chemical Sensors Group - Department of Chemistry
University of Toronto - Erindale Campus
3359 Mississauga Road
Mississauga, Ontario, CANADA L5L 1C6

Bernard Schneider
Department of Biomedical Engineering
Case Western Reserve University
Cleveland, OH 44106

Professor Willfred Schramm*
Consortium for Devel. Rep. Biology
300 N. Ingalls Street
University of Michigan
Ann Arbor, MI 48109

Bernard H. Schneider
Department of Biomedical Engineering
Case Western Reserve University
Cleveland, OH 44106

A. Z. Schreiner
Department of Chemistry - Box 8201
North Carolina State University
Raleigh, NC 27695-8201

William T. Scouten
P. O. Box 3703
Davidson, NC 28036

Professor W. Rudolf Seitz*
Department of Chemistry
University of New Hampshire
Durham, NH 03824

Satinder Sethi
Glaxo, Inc.
1035 Swabia Court, CNII Room 11
Morrisville, NC 27560

Roey Shaviv
3965 Research Park Drive
Ann Arbor, MI 48108-2296

Brian L. Shigekawa**
Organon Teknika Corporation
100 Akzo Avenue
Durham, NC 27704

Gary R. Siebert
Becton Dickinson Research Center
21 Davis Drive
Research Triangle Park, NC 27709

Jackie Stonehuerner
Department of Chemistry
Duke University
Durham, NC 27706

Maylon B. Taylor
Becton Dickinson Research Center
P. O. Box 12016
Research Triangle Park, NC 27709

Mike Thompson
Department of Chemistry
University of Toronto
880 St. George Street
Toronto, Ontario, CANADA M5S 1A1

Nancy L. Thompson
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Roy Thompson
CRDEC
Attn: SMCCR-RSB/Biotech Division
Aberdeen Proving Ground, MD 21010-5423

William A. Tilghman, Jr.
Department of Chemistry - Flanagan Building
P. O. Box 16
East Carolina University
Greenville, NC 27834

Juan L. Torres
AKZO Corporate Research America Inc.
NCSU Facility Box 8612
Raleigh, NC 27695

Mirtha Umana
Consultant
6823 Falconbridge Road
Chapel Hill, NC 27514

Professor Yoshio Umezawa*
Department of Chemistry
Hokkaido University
Kita-yu
Sapporo, 060, JAPAN

Bernard J. Van Wie
Department of Chemical Engineering
Washington State University
Pullman, WA 99164-2710

Howard C. Van Woert, Jr.
Montecito Apts.
3828-1 Colgy Drive
Raleigh, NC 27609

Elaine Vandenberg
Chemical Sensors Group - Department of Chemistry
University of Toronto - Erindale Campus
3359 Mississauga Road
Mississauga, Ontario, CANADA L5L 1C6

Frank Via
Akzo
Dobbs Ferry, NY 10522

Stephen Vigmond
Department of Chemistry
University of Toronto
80 St. George Street
Toronto, Ontario, M5S 1A1 CANADA

Dr. Jan Vleggaar
AKZO Corporate Research America, Inc.
North Carolina State University, Research Facility
P. O. Box 8612
Raleigh, NC 27695

Glen Vonk
Becton-Dickinson
P. O. Box 12016
Research Triangle Park, NC 27709

Masayoshi Watanabe
Department of Chemistry - CB 3290
University of North Carolina-Chapel Hill
Chapel Hill, NC 27599-3290

Dr. Richard Webb
Organon Teknika
100 AKZO Avenue
Durham, NC 27704

Jennifer Weist
Massachusetts Institute of Technology
56-112
Cambridge, MA 02139

Gregory A. Wemhoff
Bio/Molecular Engineering Branch
Code 6190
Naval Research Laboratory
Washington, DC 20375-5000

Carol Whisnant
Research Triangle Institute
P. O. 12194
Research Triangle Park, NC 27709

Donna J. Wiedemann
Department of Chemistry - CB 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Professor Mark Wightman*
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Professor Lemuel B. Wingard*
Department of Pharmacology
School of Medicine
University of Pittsburgh
Pittsburgh, PA 15261

Dr. David White
Institute for Applied Microbiology
University of Tennessee
10515 Research Drive - Suite 300
Knoxville, TN 37932-2567

Ching-wan Yip
Department of Chemistry
Virginia Polytechnic Institute and
State University
Blacksburg, VA 24061

Professor Jay Zemel*
Center for Chemical Electronics
Moore School of Engineering
Department of Electrical Engineering
University of Pennsylvania
Philadelphia, PA 19104

Junguo Zhao
603 D Hibbard Drive
Chapel Hill, NC 27514

*Speaker

**Session Chairman

SYMPOSIUM ON BIOSENSORS - ORGANIZING COMMITTEE AND STAFF

Dr. William E. Hatfield, Symposium Committee Chairman
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Dr. Richard P. Buck
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Dr. Edmond F. Bowden
Department of Chemistry - Box 8204
North Carolina State University
Raleigh, NC 27695

Dr. Mirtha Umana
Consultant
6823 Falconbridge Road
Chapel Hill, NC 27514

Mrs. Linda Caulder
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290

Mrs. Evelyn Kidd
Department of Chemistry - Campus Box 3290
University of North Carolina
Chapel Hill, NC 27599-3290